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Cloning and expression of *Taenia ovis* antigens in *Escherichia coli*

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Double stranded DNA complementary to poly(A)$^+$ mRNA from the tapeworm *Taenia ovis* was cut with Sau 3A to an average length of about 300 bp and inserted into the Bam HI site of the expression plasmids pEX1, pEX2 and pEX3. These plasmids express a hybrid protein derived from a fusion of the cro gene with the lac Z gene (truncated at its 5′ end by 53 bp) of phage λ. Cloning sites lie downstream from the gene fusion. *Escherichia coli* infected with another plasmid (pCI857) bearing the temperature sensitive repressor of phage λ was transformed with the pEX plasmids into which *T. ovis* DNA had been inserted; recombinants were selected by growth at 30°C in the presence of ampicillin at 100 μg ml^{-1}. Replicas were made and hybrid protein expression induced in recombinants by transferring them to 42°C. Several recombinants expressing antigenic determinants of *T. ovis* were detected with *T. ovis* infected sheep serum that had been absorbed to remove antibodies to *E. coli*. Of five selected for further study, three expressed hybrid proteins of between 165 and 170 kDa of which the *T. ovis* component contributed between 48 and 55 kDa; in the other two, the tapeworm contribution was between 0.5 and 1.5 kDa. These antigenic determinants may be of some interest with respect to vaccine development since they are expressed during the normal course of *T. ovis* infection in sheep, and they are also present in the oncosphere – the infective larva of the parasite which stimulates immunity in sheep. The native antigens in adult worms and oncospheres that correspond to the antigenic determinants produced by the recombinant clones comprise a number of species ranging from 92.5 to 180 kDa. Tests with affinity purified antibodies indicate that the expressed products of the clones represent different epitopes on the same subset of polypeptides in both adult worms and oncospheres.

Key words: Antigen; cDNA library; Expression; Plasmid vector; Sheep; *Taenia ovis*

Introduction

Larval taeniid cestodes of both mice and sheep provoke strong immunity to challenge infection in their hosts [1]. A number of studies have shown that the host-protective antigens involved are associated with the oncosphere, the infective stage for the intermediate host. In *Taenia taeniaeformis*, which infects mice, the antigens are membrane-bound [2] and they have been partially characterised by a variety of techniques [3–5]. Somewhat less is known about the corresponding oncospheral antigens of sheep parasites – *T. ovis*, *T. hydatigena* and *Echinococcus granulosus*.

There is interest in larval cestodes from the point of view of vaccine development because of all helminth parasites they generate the most effective immunity to challenge and their hosts have been successfully vaccinated with antigen preparations on numerous occasions [1,6]. However, extending these observations to the production of a practical non-living vaccine for use in the field has been hampered by the relative scarcity of the antigens involved. To seek a possible solution to this problem, recombinant DNA techniques have been investigated using *T. taeniaeformis* as a model system and a number of bacterial clones (involving the use of the phage vector λgt11) producing polypeptides of this parasite have been identified [7,8]. An artificially raised polyvalent rabbit antiserum to *T. taeniaeformis* oncospheres

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Abbreviations: HRP, horseradish peroxidase; PAGE, polyacrylamide gel electrophoresis; PBS, phosphate-buffered saline; PMSF, phenylmethylsulfonyl fluoride; SDS, sodium dodecyl sulphate; TBS, Tris-buffered saline; TCA, trichloroacetic acid; TNE, Tris-NaCl-EDTA; To1,3,5,7,8, recombinant colonies expressing *Taenia ovis* antigens.

was used principally for screening purposes; however, at least some of the antigenic products produced reacted with infected mouse serum so they are clearly related to antigens which are 'seen' by the host during the course of infection.

In the present study, DNA of the sheep parasite, *T. ovis*, has been expressed in bacteria. A plasmid rather than phage expression vector was used and the screening of recombinant bacteria for their ability to synthesise antigenic determinants of the parasite was carrried out with serum from naturally infected sheep.

Materials and Methods

Procedure. Essentially, the procedure followed was similar to that previously described [7] but a plasmid rather than phage vector was used: polyadenylated mRNA was isolated from adult *T. ovis* recovered from dogs, copied into cDNA, and the cDNA incorporated into a plasmid vector which was then used to transform *Escherichia coli*. Replicates of the transformants were screened in an immunoassay using serum from infected sheep.

Chemicals and enzymes. All chemicals were analytical grade and solutions were sterilised by autoclaving or filtration before use. Special reagents and their suppliers were as follows: primer oligo(dT_{15}) and nucleotide triphosphates (Boehringer-Mannheim) and oligo(dT_{15})-cellulose (Sigma). Enzymes were purchased from the following suppliers and used in accordance with the manufacturers' specifications: AMV reverse transcriptase, DNA polymerase 1 and calf intestinal phosphatase (Boehringer-Mannheim); RNase H (Bethesda Research Laboratories); T4 DNA ligase and the restriction enzymes BamHI and Sau 3A (New England Biolabs).

Parasites. Adult *Taenia ovis* were recovered from dogs necropsied at the Canberra and Queanbeyan dog pounds. The worms were washed thoroughly in phosphate-buffered saline pH 7.3 (PBS) and identified by hook size and proglottid morphology [9]. They were then weighed prior to storage in liquid nitrogen.

Preparation and translation of RNA. mRNA was prepared from adult *T. ovis* by the guanidinium thiocyanate procedure followed by centrifugation over a 5.7 M CsCl cushion. The poly(A)$^+$ fraction was separated by oligo(dT) cellulose chromatography and translated in an in vitro cell-free system derived from rabbit reticulocyte lysate. These methods were as described for similar studies on adult and juvenile *Fasciola hepatica* [10].

Immunoprecipitation. Immunoprecipitation and analysis of antigenic polypeptides in 20 μl samples of translation products was carried out using previously described procedures [10,11]. The serum was obtained from (i) an 18 month old virgin ewe (referred to as Ewe 5) that had received an oral infection of 3000 *T. ovis* eggs at 12 months, a 3000 egg challenge 3 months later, then two intramammary infusions [12] of *T. ovis* hatched and sonicated oncospheres, each of about 200 μg (= 25 000 oncospheres), separated by a month; and (ii) a 2-year-old virgin ewe (Ewe 3) that had been infected with 3000 *T. ovis* eggs at 12 months followed by challenge infections with the same dose of eggs 3 and 9 months later. Worm-free sheep raised in the Zoology Department provided the normal serum. Some colostrum deprived normal sheep serum was generously provided by Dr. M.D. Rickard, University of Melbourne.

The sheep sera were tested for anti-*T. ovis* oncosphere activity in a dot blot test. *T. ovis* oncosphere antigens were prepared by hatching eggs in hypochlorite [13], and then washing and sonicating the oncospheres in PBS. Protein determinations were made [14] and the protein concentration adjusted to 1.0 mg ml^{-1}. 2 μg samples were spotted on to nitrocellulose and exposed to serial two fold dilutions of test serum after blocking unreacted sites on the nitrocellulose in 5% Diploma instant skim milk powder in PBS (see below). Subsequent washing and development procedures were as described below for screening filters.

Synthesis and preparation of cDNA for cloning. Polyadenylated mRNA was converted into cDNA by the reverse transcriptase, RNAase H, and DNA polymerase 1 method [15] with 4 modifi-

cations: (1) magnesium acetate was substituted for magnesium chloride in the reaction mix; (2) oligo(dT_{15}) was used as the primer for first strand synthesis; (3) first strand synthesis was not stopped by the addition of EDTA; rather second strand synthesis was proceeded with directly by the addition of 10 μl 2 M KCl, 0.8 μl RNase H (2.5 units) and 5 μl DNA polymerase 1 (25 units) followed by incubation for 4 h at 16°C. The reaction was stopped by the addition of 4 μl 0.5 M EDTA and the cDNA was phenol extracted and precipitated with ethanol. cDNA synthesis was monitored by polyethylene-imine and agarose gel electrophoresis followed by autoradiography using standard procedures [16]. For cloning purposes the cDNA was cut with Sau 3A, phenol extracted, reprecipitated with ethanol then stored at −20°C until required. Uncut cDNA had a mean size of about 1000 bp; after cutting, its average length was about 300 bp. The cDNA in this form could be inserted directly into the Bam HI site of the plasmid vector (see below).

Cloning procedure. The vectors used (see ref. 17 for details of their construction) are referred to as pEX1, pEX2 and pEX3. Each is about 5.8 kbp and carries an ampicillin resistance gene, and a fused gene consisting of the right promoter and cro gene of phage λ linked to the β-galactosidase gene of *E. coli* which is truncated at its 5′ end where it joins a polylinker derived from the plasmid pUC8. The β-galactosidase deletion (53 bp) renders the enzyme non-functional. The polylinker is followed by stop codons in all three reading frames and transcription termination fragments derived from phage fd. Use of the family of vectors provides all three translational reading frames for DNA inserted into the polylinker.

The fused gene is placed under the control of the temperature sensitive repressor CIts 857 from phage λ. This is carried on a plasmid, pCI857, rather than on the bacterial chromosome as higher cloning efficiencies are obtained (Dr. P.G. Board, personal communication). The CI repressor enables the pEX plasmids to be amplified at 30°C; expression of the fused gene is induced by transfer to 42°C, at which temperature repression by the λ promoter is removed. The vectors thus express a cro-β-galactosidase hybrid protein of 117 kDa which is insoluble and accounts for about 25% of the sodium dodecyl sulphate (SDS)-extractable bacterial protein [17].

T. ovis cDNA cut with Sau 3A to an average length of about 300 bp was ligated into the Bam HI site in the polylinker region of pEX1, pEX2 and pEX3 using standard procedures. The Bam HI-linearised pEX plasmids were pre-treated with alkaline phosphatase to prevent self-ligation.

Competent cells of *E. coli* MC1061 infected with pCI857 were transformed with plasmids containing *T. ovis* cDNA and recombinants selected by growth on L-plates containing 100 μg ml^{-1} ampicillin at 30°C for 20 h. Replicates of colonies were made on BA45 nitrocellulose filters (Schleicher and Schuell) which were placed colony-side uppermost on fresh L-plates containing ampicillin at 100 μg ml^{-1}. After growth at 30°C for 3 h, the plates were transferred to 42°C for a further 2.5 h to obtain hybrid protein expression.

The filters were screened with Ewe 5 serum essentially as described [18] except that 5% Diploma instant skim milk rather than horse serum was used as the blocking agent. They were incubated with a 1:100 dilution of preabsorbed Ewe 5 serum (see below) for 1 h, washed, exposed to a 1:1500 dilution of rabbit anti-sheep Ig coupled to horseradish peroxidase (HRP) (Cappel Laboratories) for 1 h, washed, then incubated in HRP colour development reagent (BioRad) and 0.015% H_2O_2 for 10–20 min. Positive colonies were identified on the master plates and replated on L-agar containing ampicillin at 100 μg ml^{-1}. Further replicas were made on nitrocellulose and rescreened using both Ewe 5 and normal sera. This procedure was carried out twice. Glycerol stocks of consistently antigen-positive colonies were stored at −70°C.

Absorption of serum. Ewe 5 serum and normal sera were absorbed repeatedly to remove antibodies to *E. coli*; these are present in all animal sera and can generate unacceptable background levels of staining [19]. The bacteria were grown as a lawn on nitrocellulose sheets, lysed, and their constituent proteins renatured as described above. One 85 mm filter was used to absorb 10 ml of a

1:100 dilution of serum for 1 h at room temperature; this procedure was repeated up to four times. Absorbed, diluted sera were stored at −20°C until required.

Polyacrylamide gel electrophoresis (PAGE) and Western blotting of hybrid proteins. Hybrid proteins containing *T. ovis* antigenic determinants were analysed in a BioRad Protean Cell by PAGE, in 8% gels containing SDS [20], as well as by Western blotting [21]. A loopful of bacteria from glycerol stocks was used to inoculate 10 ml L-broth containing ampicillin at 100 μg ml^{-1}. The culture was maintained with shaking at 30°C until the optical density (680 nm) reached about 0.15. The culture was then transferred to 42°C for 2 h to induce synthesis of hybrid proteins.

2.5 ml of the culture was centrifuged for 2 min on an Eppendorf centrifuge. The pellet was resuspended in 0.5 ml 0.1 M Tris pH 7.3 containing 1 mg ml^{-1} lysozyme and 0.1% Triton X-100 and left to stand for 30 min at room temperature. After centrifugation as above the supernatant was discarded. 60 μl of 2 × SDS sample buffer and 60 μl water were added to each pellet, along with 5 μl β-mercaptoethanol, then vortexed, boiled for 2 min and centrifuged again. 30 μl samples were loaded onto gels which were run at 40 mA for 3–4 h. Molecular weight markers were included on each gel along with samples from bacteria infected with one of the parent plasmids (pEX1) and a recombinant that was negative in the antibody screening procedures.

When Western blots were performed, gels were set up in a transblot apparatus (BioRad) and the proteins transferred to nitrocellulose overnight. The nitrocellulose was then probed with Ewe 5 or normal serum as described above for filters except that DNAse I treatment was omitted. In addition, Western blots were also probed with an antiserum to sonicated oncospheres prepared in BALB/c mice. These mice received 100 μg of sonicated *T. ovis* oncospheres in Freund's complete adjuvant i.m. into the thighs on 3 occasions, 2 weeks apart, prior to bleeding out and preparing serum. This serum had a titre of about 1/10000 in a dot blot performed as described above for Ewe 5 serum except that the second antibody was goat anti-mouse Ig coupled to HRP (BioRad).

Purification of hybrid proteins. 250 ml cultures of recombinants were grown and induced as described above. Cells were harvested by centrifugation and resuspended in TNE (5 mM Tris-HCl, pH 7.5, 150 mM NaCl, 12% sucrose, 5 mM EDTA and 1 mM phenylmethylsulfonyl fluoride (PMSF)). They were frozen, thawed and sonicated, frozen and thawed again, then treated with lysozyme (250 μg ml^{-1}), DNAase and RNAase (both at 20 μg ml^{-1}) for 20 min at 0°C. The freeze-thaw-sonicate procedure was repeated and then Triton X-100 added to 10% (w/v). After 10 min at 0°C, the extracts were spun over a cushion of 40% sucrose at 21800 × *g* for 20 min. The pellet was washed twice in TNE plus sucrose then resuspended in 10 mM Tris-HCl, pH 7.4, 8 M urea. Following centrifugation at 21800 × *g* for 10 min, the supernatant was retained and the pellet re-extracted. Urea was removed by dialysis against PBS. The hybrid proteins remained in solution following this procedure.

Affinity purification of antibodies to hybrid proteins. Antibodies to hybrid proteins in Ewe 5 serum were affinity purified on 8 cm nitrocellulose filters essentially as described [8]. Filters were exposed separately to 1.0 mg of each hybrid protein in 5 ml PBS for 1 h, washed, blocked, then incubated with 1.0–2.0 ml Ewe 5 serum diluted 1:15 for 3 h. Bound antibodies were eluted with 5 ml 0.1 M glycine-HCl, 0.15 M NaCl, pH 2.5, for 1 min. Following restoration to pH 8.0 with 2.0 M Tris-HCl, antibody samples were stored at −20°C until required.

Native antigens corresponding to cloned antigens. Affinity purified antibodies to hybrid proteins were used to probe antigen preparations from adult worms and oncospheres of *T. ovis* in order to characterise the native antigens corresponding to the cloned antigens. Western blots were performed essentially as above, but to amplify signals, a third antibody, goat anti-rabbit HRP (BioRad), at 1:500 dilution, was used for 1 h prior to colour development. The antigen preparations were as follows: about 10^7 *T. ovis* eggs (supplied by S.B. Lawrence) were hatched in hypochlorite [13], washed and sonicated in 5 ml PBS in the presence of 2 mM PMSF (to inhibit proteases).

Aliquots of 200–250 μg ml^{-1} were stored at −20°C until required. Adult worms were homogenised in TBS (20 mM Tris-HCl pH 7.5, 500 mM NaCl, 5 mM EDTA) containing 1 mM PMSF, clarified by centrifugation at 13000 × *g* for 10 min and aliquots of the supernatant (about 1 mg ml^{-1}) stored at −20°C. The pellet was also extracted twice with TBS containing 5% SDS. Supernatants were combined to give an SDS-soluble protein extract).

Between 40 and 80 μg samples of antigen were run on 10% SDS-PAGE gels, and Western blots were probed separately with affinity purified antibodies to each of the 5 recombinant hybrid proteins.

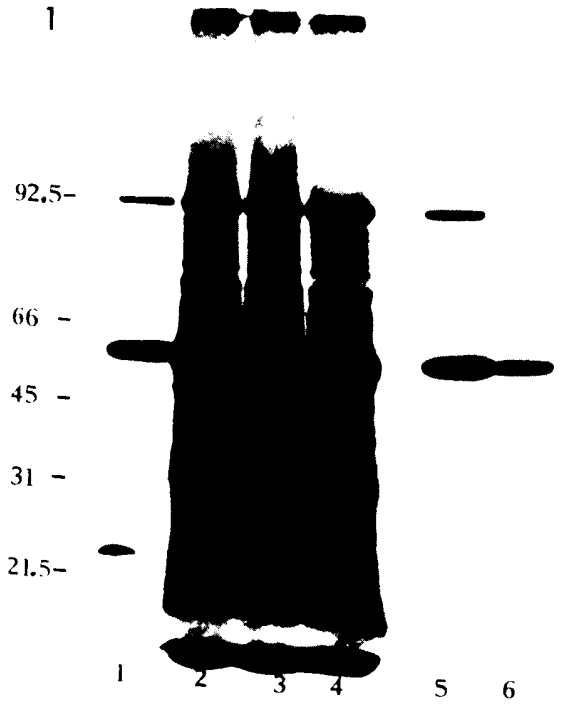

Fig. 1. In vitro translation products of *T. ovis* adult worm RNA, labelled with [^{35}S]methionine, immunoprecipitated with sheep serum and examined by SDS-PAGE followed by autoradiography. Lane 1: translation products immunoprecipitated by Ewe 5 serum. Lanes 2–4: translation products from three different RNA samples. Lane 5: translation products immunoprecipitated by Ewe 3 serum. Lane 6: translation products precipitated by normal serum. MW markers (× 10^3) are shown.

Results

Isolation and translation of mRNA. About 1% of the wet weight of *T. ovis* processed by the guanidinium thiocyanate method was recovered as RNA; almost 2% of the total RNA was eluted from oligo(dT) cellulose columns as poly(A)$^+$ mRNA.

Incorporation of [^{35}S]methionine into trichloroacetic acid (TCA)-precipitable translation products was stimulated 3–30-fold above background levels by total RNA from *T. ovis*. Differing degrees of incorporation were obtained with different RNA samples. Despite this, however, the optimum amount of RNA in the reaction was consistently 0.4 μg.

The range of polypeptides synthesised in vitro by samples of RNA from three different adult worms is shown in Fig. 1 (lanes 2–4). Polypeptides covering a wide range of molecular weights were synthesised, with two (92.5 and 58 kDa) especially prominent.

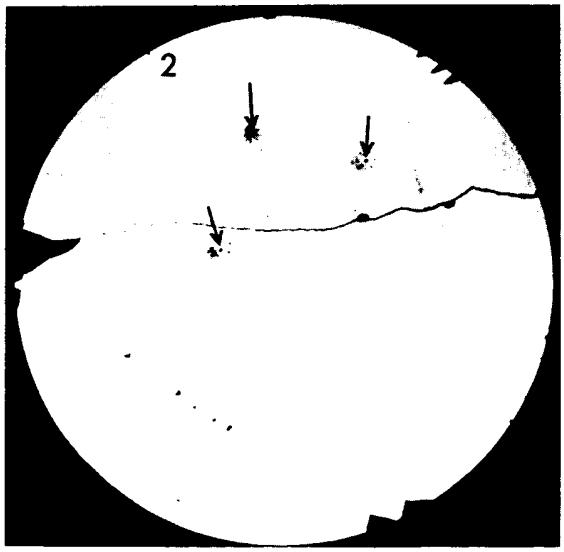

Fig. 2. Nitrocellulose replica of part of a *T. ovis* cDNA library in *E. coli* MC1061 which had been induced by growth at 42°C for 2 h then screened with Ewe 5 serum (absorbed four times) followed by rabbit anti-sheep Ig coupled to HRP. Positive colonies are arrowed; negative colonies are barely visible. Each positive colony is ringed by a set of reference marks.

Immunoprecipitation of translation products. Ewe 5 serum had a titre of 1:1024 when tested in a dot blot against 2 μg *T. ovis* oncospheral antigens; that of Ewe 3 was 1:512.

Serum from a worm free sheep absorbed one polypeptide in particular (58 kDa species) from the translation products (Fig. 1, lane 6). However, both infected sheep sera (Ewe 3 and Ewe 5) specifically immunoprecipitated polypeptides of 92.5 kDa and 29 kDa (Fig. 1, lanes 1 and 5). In addition Ewe 5 serum immunoprecipitated a 23 kDa polypeptide. A few faint bands are detectable in the infected sheep sera tracks (ranging from about 65 to 70 kDa) but the amounts are insignificant compared with the others.

Cloning cDNA and immunological screening of recombinants. About 50000 recombinant colonies were obtained from 100 ng of Sau 3A-cut *T. ovis* cDNA ligated into the Bam HI site of the vectors. Control transformations using phosphatased vectors alone did not give rise to background colonies.

After inducing hybrid protein expression at 42°C and screening with pre-absorbed Ewe 5 serum, a few colonies on each plate gave positive signals (Fig. 2). Positive colonies on the master plate were identified, hand-picked and rescreened (Fig. 3) with both absorbed Ewe 5 and normal sera to characterise consistently positive colonies.

Nature of the expressed hybrid proteins. Induced bacterial extracts were examined by SDS-PAGE. The cro-lac fusion protein expressed by the parental vectors (pEX1, 2 and 3) has a molecular weight of 117 kDa (Fig. 4, lane 8). Three of the recombinants (referred to as To1, To3 and To5) expressed hybrid proteins of around 165 to 170 kDa (Fig. 4, lanes 5, 6 and 7) of which the *T. ovis* component was between 48 and 55 kDa. Two (To7 and To8 in lanes 4 and 3 of Fig. 4, respectively) expressed hybrid proteins only slightly larger (by 0.5–1.5 kDa) than the parental type (lane 8). A recombinant that was negative in the immunological screening of the library is also included on this gel (lane 2).

When replicas of these gels were transferred to nitrocellulose by Western blotting and screened

Fig. 3. Rescreen of positive colonies handpicked from master plates (see Fig. 2) onto fresh plates and then replicated onto nitrocellulose filters. Ewe 5 serum (absorbed four times) was used for screening.

with either Ewe 3 or Ewe 5 sera (both absorbed twice) only the bands corresponding to the expressed hybrid proteins of To1, To3, To5, To7 and To8 were detected (Fig. 5). The parental cro-lac protein and that of a negative recombinant did not react in the test. A similar replica probed with mouse anti-*T. ovis* oncosphere serum (at a 1:10 dilution) and goat anti-mouse Ig coupled with HRP (at 1:1500) stained the hybrid protein bands of the 5 recombinants which had reacted positively with Ewe 3 and Ewe 5 sera. Negative results were obtained with appropriate normal sera.

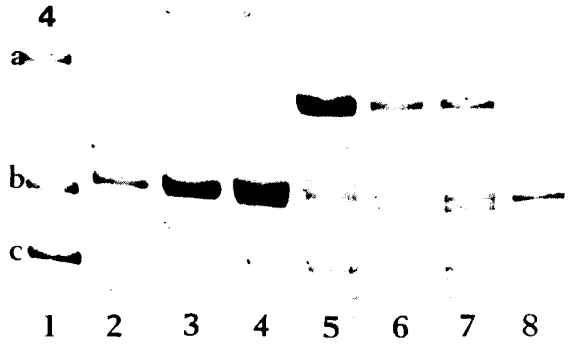

Fig. 4. SDS-PAGE of bacterial extracts prepared from parental and recombinant colonies containing *T. ovis* cDNA. Only the upper portion of the gel is shown. Lane 1: molecular weight markers ($\times 10^3$): a, myosin (200); b, β-galactosidase (116.25); and c, phosphorylase B (92.5). Lane 2: from recombinant colony, but negative in the screening with Ewe 5 serum. Lane 3: from recombinant colony To8. Lane 4: from recombinant colony To7. Lane 5: from recombinant colony To5. Lane 6: from recombinant colony To3. Lane 7: from recombinant colony To1. (Lanes 3–7 were extracts derived from colonies that reacted positively with Ewe 5 serum in the initial screen and rescreen as shown in Figs. 2 and 3.)

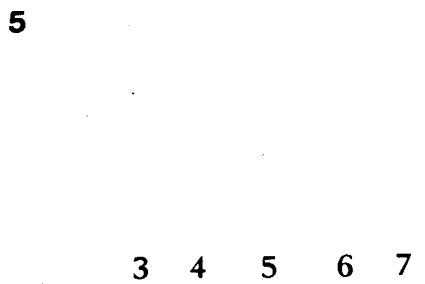

Fig. 5. Western blot of a replica gel of that shown in Fig. 4, probed with Ewe 5 serum (absorbed twice). Only the hybrid protein bands of To1, To3, To5, To7 and To8 are positive. The lanes correspond to those shown in Fig. 4.

Native antigens corresponding to cloned antigens. Affinity purified antibodies to each of the partially purified hybrid proteins were tested in Western blots. From Fig. 6, it is clear that affinity purified antibodies to each of To1, To3 and To5 reacts with all three of these hybrid proteins Some degradation of each hybrid protein appears to have taken place during purification procedures as evident by a number of bands of slightly lower molecular weight than the intact protein. In the case of To7 and To8, affinity purified antibodies showed less cross reactivity towards each other and none towards To1, To3 and To5.

The native antigens corresponding to the cloned antigens are found in antigenic extracts of both adult worms and oncospheres (Fig. 7). Affinity purified antibodies to each of the hybrid proteins reacted with two antigens, of between 110 and 118 kDa in all cases. In addition, each antibody reacted with two higher molecular weight species (150 kDa and 180 kDa) and two slightly smaller polypeptides (100 kDa) in adult worm extract. One antigen (100 kDa) in oncospheres reacted with affinity purified antibodies to To1 and To3 only. Inspection of lanes 2 and 3 (Fig. 7) indicates a number of minor bands are also present in the 92.5–100 kDa range. Tests with the SDS-soluble protein extract of adult worms suggest the 110 and 118 kDa species are to some extent membrane bound. Comparison with Fig. 8 indicates that the above antigenic polypeptides represent a small subset of the total number of polypeptides detectable in adult worm and oncospheral antigen preparations by Coomassie blue staining and Western blotting.

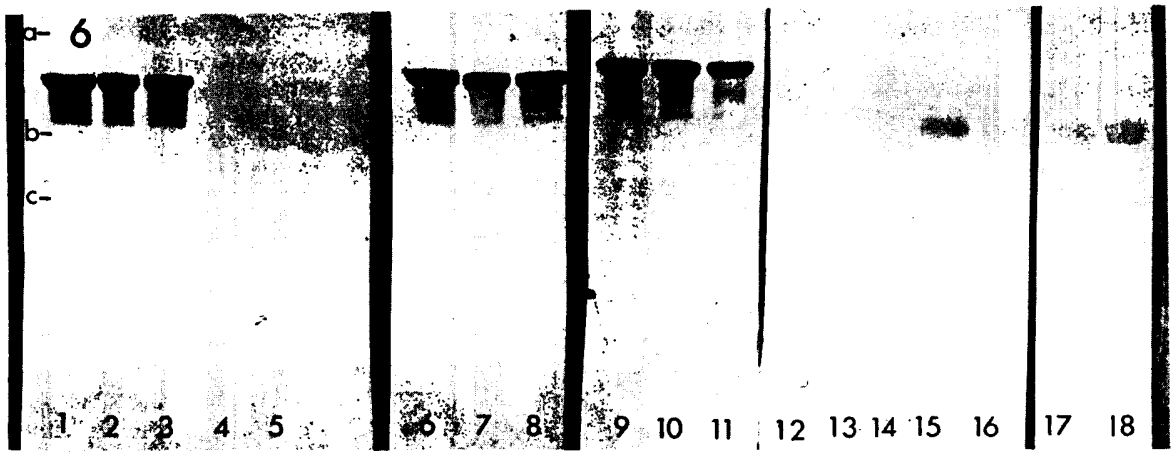

Fig. 6. Immunoreactivity of affinity purified antibodies to partially purified cloned antigens (hybrid proteins) extracted from recombinants To1, To3, To5, To7 and To8 as tested by Western blotting. Lanes 1–5: extracts from To1 (lane 1), To3 (lane 2), To5 (lane 3), To7 (lane 4), To8 (lane 5), probed with affinity purified antibody to To1. Lanes 6–8: extracts from To1 (lane 6), To3 (lane 7), To5 (lane 8) probed with antibody to To3 (the negative lanes corresponding to lanes 4 and 5 are not shown). Lanes 9–11: as for lanes 6–8 but probed with antibody to To5 (the negative lanes are not shown). Lanes 12–16: as for lanes 1–5 but probed with antibody to To7. Lanes 17,18: extracts from To7 (lane 17), To8 (lane 18) probed with antibody to To8 (the negative lanes corresponding to lanes 12, 13 and 14 are not shown). (MW markers a,b,c, as in Fig. 4.)

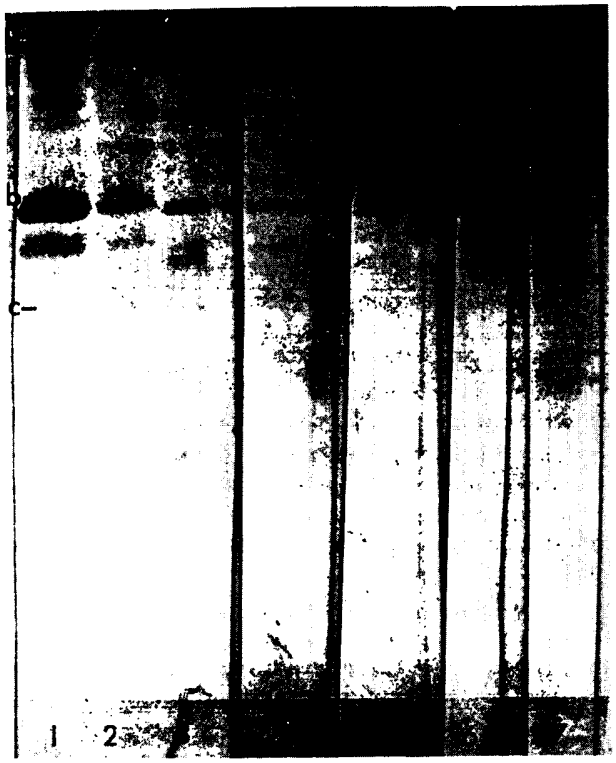

Fig. 7. Western blots of *T. ovis* adult worm and oncospheral extracts probed with affinity purified antibodies to cloned antigens from recombinants To1, To3, To5, To7 and To8. Lanes 1,2 and 3: *T. ovis* adult worm soluble extracts, SDS extract of adult worms and oncospheral extract respectively probed with antibody to To1. Lane 4: oncospheral extract probed with antibody to To3. Lane 5: as for 4, antibody to To5. Lane 6: as for 4, antibody to To7. Lane 7: as for 4, antibody to To8. (Note: Reactivity of each affinity purified antibody towards adult worm soluble extract (lane 1) and SDS extract of adult worms (lane 2) was comparable in every case and the results are not shown.) (MW markers a,b,c as in Fig. 4.)

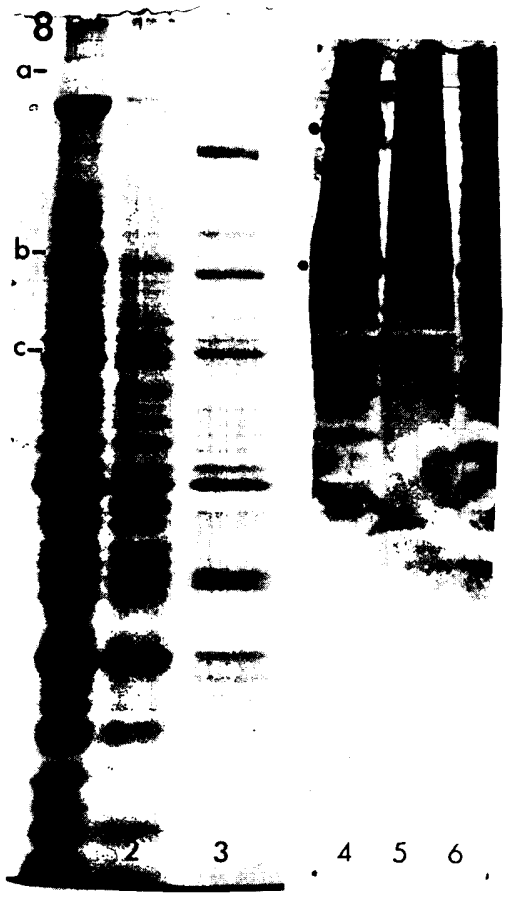

Fig. 8. Lanes 1–3. 10% SDS-PAGE gel of *T. ovis* proteins stained with Coomassie blue. Soluble extract of adult worms (lane 1), SDS-solubilised material from adult worms (lane 2), and oncospheres (lane 3). Lanes 3–5: Western blot of replica of Coomassie blue stained gel (lanes 1–3) probed with Ewe 5 serum (from which antibodies used in Figs. 6 and 7 were affinity purified). The bands marked • correspond to the prominent bands of 110 kDa and 180 kDa in lane 1. (MW markers a,b,c as in Fig. 4.)

Discussion

The present study succeeded in cloning and expressing DNA of a larval tapeworm parasite of sheep, *T. ovis*, in *E. coli*. Moreover, the recombinants produce antigens that are expressed by oncospheres during the course of infection in sheep. The techniques followed conventional gene cloning strategies except that a plasmid rather than phage expression vector system (which has been used with other flatworms) was employed.

Antigens of oncospheres were the principal focus of this investigation because of their ability to provoke immunity in the intermediate host [1]. The rationale for using adult worms as a source of mRNA coding for them was as follows: all stages of oncospheral development are found along the length of the strobila and accordingly the appropriate mRNA coding for these antigens must be present in the strobila. Some species of mRNA prepared from adult *T. ovis* coded for polypeptides which were recognised as antigenic by infected sheep serum. However, whether these polypeptides represent host protective antigens or

at least epitopic regions of them remains to be determined. Indeed, there is a clear need for a more detailed antigenic characterisation of *T. ovis* along the lines that have been followed with *T. taeniaeformis* [3–5].

cDNA prepared from poly(A)$^+$ RNA of *T. ovis* was cloned and expressed using the pEX series of vectors [17]. The cDNA was cut with Sau 3A prior to cloning so it is assumed that full length copies were not processed. It has been found [17] (P.G. Board, personal communication) that it is not essential for full length cDNA segments to be cloned in order to preserve the antigenicity of expressed products. Provided a short segment of amino acids is folded correctly (a likely event since the cDNA is inserted downstream of the large cro-lac Z DNA segment and is expressed in association with it) the epitope will be recognised by antibodies in the serum used for screening.

The *T. ovis* antigenic determinants produced by the recombinants (To1, To3, To5, To7 and To8) are clearly 'seen' by sheep during the course of infection. This is borne out by their recognition by antibodies in Ewe 3 serum, an animal that had only been exposed to oral challenges with *T. ovis* eggs. That the determinants are also found in oncospheres was confirmed by screening with an anti-*T. ovis* oncosphere serum raised in mice. These observations are therefore of some interest, especially in the context of vaccination against larval cestode infection, since oncospheral antigens are likely to be essential ingredients of any vaccine [1].

Of the 5 antigen-positive recombinants that were examined by SDS-PAGE and Western blotting, 2 produced hybrid proteins only slightly larger than that produced by parentals, while 3 produced hybrid proteins 49–55 kDa larger than the parentals. It would seem, therefore, that the former contain recombinant plasmids with short coding sequences of *T. ovis* (50–100 bp) whereas the latter contain inserts of around 1500 bp. These values represent the extremes in the size range of the Sau 3A cut cDNA. More common intermediate sizes of cDNA have not appeared among recombinants isolated to date.

The antigenic determinants produced by To1, To3 and To5 are immunologically related since affinity purified antibodies to each react with all three. This does not mean that the cloned antigens are necessarily identical, but rather share epitopes with each other. Sequencing data will be required before their degree of relatedness can be established. To7 and To8 showed less cross reactivity between each other; and nucleotide sequence information now available for these two clones (unpublished data) shows no homology exists between them. Thus, antigenic cross reactivity would be unexpected.

The affinity purified antibodies to each of the hybrid proteins reacted with a similar subset of polypeptides in a soluble antigen extract of adult worms and to a more limited number of polypeptides in an SDS extract of insoluble worm material and sonicated oncospheres. These results are interesting for a number of reasons: (1) antigenic determinants produced by To7 and To8 apparently correspond to different epitopes on the same polypeptides; (2) those produced by To1, To3 and To5 do not cross react with To7 and To8 yet affinity purified antibodies to them appear to react with the same native polypeptides as To7 and To8; they must therefore represent different epitopes to those produced by To7 and To8 but they are clearly present on the same polypeptides as them; (3) since affinity purified antibodies to each of the hybrid proteins detected several antigenic polypeptides common to both adult worms and oncospheres, the same epitopes appear to be common to a number of antigens. This raises the possibility that some of the antigens of *T. ovis* may belong to a multi-gene family, a possibility that could be tested by isolating and characterising genomic equivalents of the cDNA clones. Alternatively, the presence of a number of immunologically related polypeptides of differing molecular weight could be indicative of a number of processing steps in the synthesis of mature antigens. Clearly, further work will be necessary to answer these questions and to determine whether the expressed products can be readily purified and used to protect sheep against infection with *T. ovis*.

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